Vol. VI. *January, 1904.* *No. 2*

BIOLOGICAL BULLETIN.

FORM-REGULATION IN CERIANTHUS, III.

THE INITIATION OF REGENERATION.

C. M. CHILD.

In the first paper of this series ('03*a*) the typical course of regeneration in a cylindrical piece was described; in the second paper ('03*b*) some of the factors influencing the process of regeneration as a whole were discussed; these papers have served to clear the ground for a detailed analytical study of the process of regeneration in *Cerianthus* in its various manifestations. In this and following papers of the series various phases of this subject will be considered.

CHANGES IN FORM CONSEQUENT UPON SECTION.

The reduction in size of the opening at the end of a cut piece by the bending inward of the cut margins was described in its simplest form in the first paper of this series. A somewhat more general consideration of this peculiar process is necessary before proceeding to the discussion of other points.

Early in the course of my experiments upon *Cerianthus* it was noted that in nearly every case, however the pieces might be cut, the body-wall became rolled or folded in such a manner that the opening into the enteric cavity resulting from the cut was much reduced in size or was closed by approximation or contact between different parts of the body-wall. The usual result of the infolding is the complete removal of the entodermal surfaces from contact with the external water, *i. e.*, the piece rolls up or closes in such manner that the entoderm is on the inside. For convenience we may designate inrolling about a transverse axis as transverse inrolling, and inrolling about a longitudinal axis as longitudinal inrolling.

At first glance this process appears much like an adaptive reaction. In some cases it is almost as if the animal or part were

consciously closing the artificial openings. In the following paragraphs the principal forms of inrolling in the cut pieces are described.

The case of the closure of the ends of a cylindrical piece which was described in the first paper is the simplest of all. Collapse occurs with the escape of the water from the enteron and within

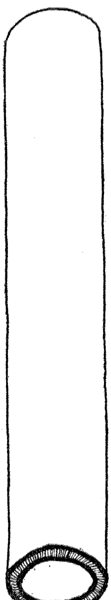

FIG. 1.

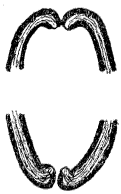

FIG. 2.

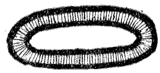

FIG. 3.

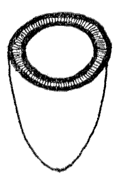

FIG. 4.

FIG. 5.

FIG. 6.

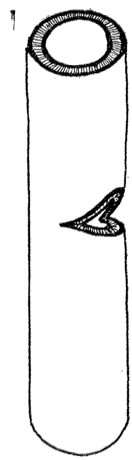

FIG. 7.

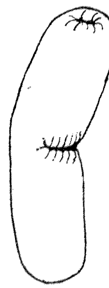

FIG. 8.

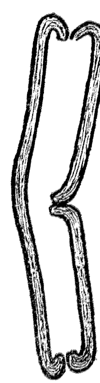

FIG. 9.

a few moments the cut ends begin to bend inward and finally close the openings except for the small slits between the folds. The diagrams, Figs. 1,[1] 2 and 3, illustrate this case, Fig. 1 representing the cylindrical piece at the time of section, Fig. 2 the longitudinal section of the ends after the bending in of the cut margins, and Fig. 3 a transverse section, indicating the flattening

[1] The diagrams representing the inrolling are much less highly magnified than preceding figures.

of the piece as it lies on a flat surface. In pieces of this kind complete collapse and contact of the body-walls is prevented by the large mass of mesenteries and mesenterial filaments which occupy the enteron. These are not represented in the figures, but they fill the whole enteron after collapse. Any solid mass in the enteron would of course have the same effect.

A piece cut from the extreme aboral end of the body (Fig. 4) differs in certain respects from the piece just described. Figs. 5 and 6 show the changes in a piece of this kind. Here the cut end becomes rolled inward to a much greater extent than in the previous case so that the enteron is nearly filled by the inrolled portion and the cut surface is so situated that closure by growth of new tissue from this surface is impossible. The reason for the greater degree of rolling in this piece as compared with the longer piece is undoubtedly to be found in the absence of mesenteries, except a single pair, in the aboral region. Since the enteric cavity is not filled with a mass of mesenterial filaments as in a region further orally the inrolling continues until the entire cavity is practically obliterated by the inrolled parts.

If a cut be made in one side of the body or a piece removed as in Fig. 7, the cut edges roll inward as in other cases, but in addition to this the body becomes bent at the level of the cut, so that here also the inrolled edges are brought into contact (Figs. 8 and 9).

The widest departures from the typical form are found, however, in those pieces which were cut longitudinally as well as transversely. In these the results differ to some extent according to the shape and relations of the pieces. Fig. 10 represents a cylindrical piece split longitudinally on one side. One form after collapse and inrolling of cut margins is shown in Fig. 11. Fig. 12 represents a transverse section and Fig. 13 a longitudinal section of one end. In Fig. 14 another form of closure is represented; here the ends fold over to a greater extent so that the opening is entirely on one side of the piece. Fig. 15 represents a transverse section of this piece and Fig. 16 a longitudinal section in the plane indicated by the vertical line in Fig. 14.

In most cases, however, the right and left longitudinal cut edges do not roll inward with equal rapidity and the result is

that the piece rolls up spirally on its longitudinal axis. Such a case is shown in Fig. 17; here the inner and outer coils of the spiral are on the same level at the outer end of the piece. Fig. 19 shows a spirally coiled piece in which the inner coils are higher than the outer. Figs. 18 and 20 represent longitudinal sections of one end of these pieces and Fig. 21 a transverse section.

The Figs. 11–21 represent only the chief types resulting from pieces like Fig. 10. All possible intermediate forms and modifi-

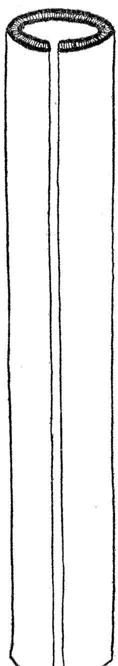

FIG. 10.

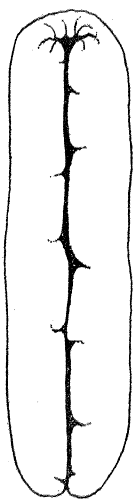

FIG. 11.

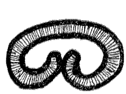

FIG. 12.

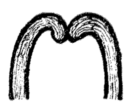

FIG. 13.

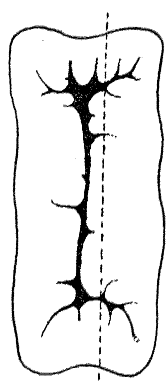

FIG. 14.

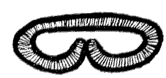

FIG. 15.

FIG. 16.

cations of these different types occur, the differences depending on various conditions, but chiefly on the relative rapidity of the inrolling in the different directions.

Semi-cylindrical pieces or longitudinal strips may roll either longitudinally or transversely. The greater the length and the less the breadth of the strip the more likely it is to roll transversely. Figs. 22–24 show a strip and two forms of transverse rolling which it may undergo. The longitudinal strips

often roll longitudinally soon after section, but by gradual inrolling of the ends finally become rolled transversely.

Loeb ('91) has suggested that the cut edges roll inward because the inner layers of the body-wall are stretched to a greater degree than the outer layers; this view assumes that all layers are more or less similar in elasticity and therefore that the layer that is most stretched will undergo the greatest contraction when the tension ceases. It is difficult to understand why one layer of the body should be more stretched than another, since all have been subjected to the same conditions, viz., the tension resulting from the fluid pressure on the walls of the enteron.

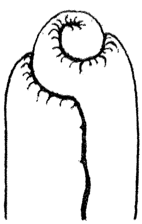

FIG. 17.

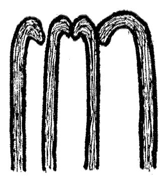

FIG. 18.

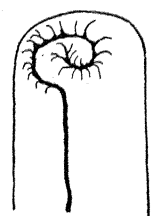

FIG. 19.

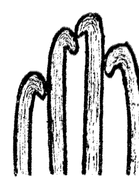

FIG. 20.

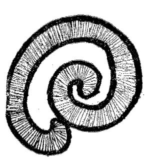

FIG. 21.

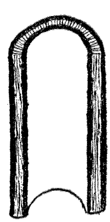

FIG. 22.

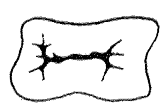

FIG. 23.

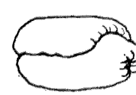

FIG. 24.

I am inclined to believe that this remarkable capacity for rolling which the pieces exhibit is due primarily to a difference in elasticity between the different layers of the body-wall, though it may be increased or modified by other factors. The succession of layers in the body-wall is as follows: ectoderm, longitudinal muscles, mesoglœa, entoderm. The mesoglœal layer is fibrillar in appearance and, while not as thick as the muscular layer, is well developed.

Judging from the fact that this layer is not folded or wrinkled, even in strongly contracted animals, the inference that it possesses

a considerable degree of elasticity appears justifiable. Under normal conditions the body-wall is subjected to tension. By section of the body at any point the internal pressure is removed and collapse occurs; the body-wall is no longer under tension and contraction of the elastic layer begins. If the ectoderm and muscles are to a large extent passive in this elastic contraction the result will be not simply a reduction in surface area, but an inrolling of the body-wall, since the mesoglœa is situated near its inner surface.

The fact that the region near the cut surface is always more strongly rolled than other parts may perhaps be the result of the direct injury to the tissues in this region, causing contraction, but here as elsewhere the contraction must be greater in the inner portions of the body-wall than in the outer, otherwise inrolling could not occur. More probably, however, the greater degree of inrolling near the cut surface is largely, if not wholly due to the fact that the physical obstacles to the inrolling offered by resistance of other tissues, etc., are much less near a free end or cut surface than elsewhere and the effect of elasticity is therefore greater. It appears probable from the preceding considerations that the mesoglœa plays the chief part in the inrolling about the cut surface as well as in regions distant from it.

Objection to this view may, however, be made on the ground that a tonic muscular contraction resulting from the injury is not only a possible but a much more probable cause of the inrolling. A brief consideration of the facts is sufficient to show that the inrolling cannot be explained as the result of muscular contraction.

This is evident first from the fact that it occurs in all directions, longitudinally and obliquely as well as transversely. If it were the result of muscular contraction we should expect it to occur only transversely since the body-wall contains only longitudinal muscles. It is difficult to understand how the contraction of longitudinal muscles could account for the inrolling of a longitudinal cut margin, since the muscle fibers are parallel to the cut. Moreover, there is no apparent reason why the inner portions of the muscular layer should contract more strongly than the outer, since all must be equally affected by the injury. And finally, observation renders it very evident that the inrolling is

not due to muscular contraction, for although strong contraction occurs at the time of section the muscles relax within a few moments and before inrolling begins. Moreover, the muscular contraction consequent upon stimulation of pieces after inrolling has occurred causes in most cases a more or less complete unrolling, provided the inrolling was in the transverse direction but does not affect inrolling in the longitudinal direction. In the light of these facts it is difficult to escape the conclusion that the inrolling is caused by some part of the body-wall axial to the muscular layer, viz., either entoderm or mesoglœa. The delicate cellular layer of entoderm cannot be supposed to possess any such elasticity; there remains therefore only the mesoglœa.

It now remains to consider whether the different forms of inrolling described and figured above are all explicable on the basis of elasticity of the mesoglœa. For this purpose we may regard the tension as resolved into longitudinal and transverse components.

As regards the inrolling of cylindrical pieces with transverse cut margins (cf. No. 1 of these studies, '03*a*, also Figs. 1 and 2 of the present paper), it is easy to see that it can proceed only a certain distance. Since the elastic tension is present in all parts of the cylindrical piece reduction in size may occur, but the cut ends are the only regions where marked change of form can take place. These are bent inward until the more prominent folds come into contact and the size of the opening is reduced. Beyond this the inrolling cannot go since contact between different parts of the margin and the radial folds into which the contracting margin is thrown both oppose further change. The appearance of the radial folds requires a word of explanation. Their presence would seem at first glance to indicate that elastic tension exists only in the longitudinal direction. A brief consideration will show, however, that this is not the case. After escape of the water from the enteron and collapse of the body, reduction of the circumference occurs throughout the whole piece, undoubtedly in consequence of the elasticity of the body-wall. There is, however, no physical ground for greater contraction in the transverse direction at the ends than elsewhere since there is no break in the transverse continuity of the body-

wall. The longitudinal component must cause inrolling at the cut end until either the local tension due to the formation of radial folds in consequence of the inrolling or the mutual contact of appressed portions of the inrolled wall opposes a resistance equal to the elastic tension. It is probable that in the oral half of the body, the mass of mesenteries and mesenterial filaments also oppose more or less resistance to the inrolling margins.

The flattening of the piece which often occurs (Fig. 3) is simply the result of gravity. If the collapsed piece lies on one side during several hours the weight of the body-wall is sufficient to bring about the flattening to a greater or less extent. In consequence of the flattening the openings at the ends are frequently elongated in the plane of flattening and slit-like, the inrolling occurring chiefly on the two margins of the slit.

In pieces from the extreme aboral end the inrolling at the cut margin may proceed much further (Figs. 4–6). Here the body-wall and especially the muscular layer is much thinner and must offer much less resistance to the elastic tension. Moreover, the enteron is practically empty in this part of the body. In consequence of these conditions the inrolling may proceed so far that portions of the margin are directed orally (Fig. 6).

The closure of a lateral cut by bending of the whole piece (Figs. 7–9) especially resembles a definite adaptive reaction, but can be explained as the result of elastic contraction. A cylindrical piece such as Fig. 1 does not become bent or curved so long as elastic tension on opposing sides of the body is equal. If in any way the tension on one side be reduced in effectiveness the body must bend toward that side. A transverse cut through the body-wall on one side, or the removal of a piece as in Fig. 7 interrupts the continuity of the body-wall. The longitudinal component of the elastic tension acts on the parts above and below the cut and causes contraction and inrolling of their edges. But by removal of a piece of the body-wall an open space is left and the longitudinal component of tension on the opposite side of the body causes bending of the piece so that the concave surface is on the side of the cut. The larger the piece cut out from the one side the greater will be the bending since it will continue until contact between the cut margins affords a resistance equal to the opposing tension.

Figs. 10–21 require little explanation. Here transverse continuity is interrupted by a longitudinal cut on one side. The form of the piece after inrolling is at least in large part a matter of chance, being dependent upon the relative rapidity with which the different margins roll inward.

In Fig. 11 the inrolling at the ends has been less than in Fig. 14 and the resulting form is different. In Figs. 17–21 the spiral form is due simply to the fact that one longitudinal cut margin rolled inward somewhat more rapidly than the other. An oblique spiral results from more rapid inrolling of the longitudinal margin near one end. It is clear that various conditions such as the degree of contraction of the muscles of a certain part of the body, the resistance offered by the mesenteries, the position of the piece in the aquarium, etc., may constitute conditions affecting the result.

The frequent rolling about a transverse axis of longitudinal strips cut from the body is clearly the result of the predominance of the longitudinal component of tension. It is interesting to note that this transverse rolling occurs only when the muscles are fully relaxed. If the piece be stimulated sufficiently to cause strong muscular contraction more or less complete unrolling often occurs. Pieces of this sort frequently roll about a longitudinal axis after cutting while the muscles are more or less contracted and then as the muscles relax after a longer or shorter time begin to roll transversely and continue until completely rolled up in a single or double spiral.

In cases of spiral or transverse inrolling (Figs. 17, 19, 23, 24) there is little resemblance to an adaptive reaction. As will appear, typical regeneration is impossible in these cases. Since it is scarcely to be supposed that in pieces of a certain form the reaction is adaptive in nature while in pieces of other forms it is due merely to elasticity it is preferable at least to attempt to analyze the apparently adaptive reaction. In the present case I think the analysis has demonstrated that the various methods of inrolling are all explicable on the basis of elastic contraction of the mesoglœa. The apparently adaptive character of the inrolling in cylindrical pieces where it results in more or less perfect closure of the ends is due to the particular physical conditions

present in such pieces. The inrolling of pieces after section is not then a definite reaction adapted to close the wound, except in so far as we may regard the presence of an elastic layer in the body-wall as an adaptation.

After the inrolling is completed gradual reduction in the size of the whole piece continues until the artificial openings are closed by new tissue or otherwise and the water pressure is again established in the enteron. This reduction in size can scarcely be due to the loss of tissue in the absence of food, for that is much less rapid. The piece appears to contract continuously after collapse and closure and if the closure and distention with water is prevented in any way, becomes much reduced within a few days. Frequently new wrinkles or folds appear as the contraction progresses, indicating that it is not due to actual loss of material but to some other cause.

There can be no doubt that this reduction in size of collapsed pieces is simply a continued reduction in the surface area of the tissues resulting from mechanical conditions. It is due at least in part to the elasticity of the body-wall (or especially of the mesoglœa). This being effective in all directions must cause gradual reduction in size of the whole after the inrolling of the margins is completed, unless it is counterbalanced in some way, which is not usually the case. This quality of the body-wall is remarkable; pieces kept under conditions where distention with water is impossible often contract to half the size after section and collapse. If they are then permitted to close and become distended with water they may again attain in two to three days almost the original size, provided the period during which they remained contracted was not too long. The longer the period of collapse the slower and less complete is the return to the original size. These facts indicate that in the absence of the tension due to internal water-pressure the tissues gradually rearrange themselves in accordance with the altered physical conditions. There is no return to the "normal" form unless mechanical conditions once more become normal.

In his study of *Cerianthus*, Loeb ('91) has attempted to explain the collapse of tentacles and other phenomena by loss of turgor in the cells. As I shall show in a later paper, this explanation is

wholly incorrect. The question as to whether osmotic phenomena play a part in the changes above described requires, however, a moment's consideration. As regards the inrolling after section and the reduction in size of the collapsed pieces there is certainly no reason for supposing that it is due to changed osmotic conditions. It is difficult to understand how, in a form like *Cerianthus* section of the body-wall at one level should cause changes in turgor in the cells of the whole piece or of those at a distant region unless we suppose that special stimuli producing these changes arise from the region of the cut. If this be the case then the change is not primarily osmotic but reactive. Moreover, the phenomena are so obviously due to elasticity that the search for any other explanation is clearly unnecessary.

The Rôle of the Slime Secretion in the Closure of the Enteric Cavity After Section.

As has been shown, the inrolling of the margins of the piece under ordinary conditions approximates the various parts of the cut surface, and thus reduces the size of the opening. The radiating wrinkles and folds into which the inrolling portions are thrown and the frequent protrusion of parts of the mesenteries through the opening render the closure by contact imperfect. There are always slits and angles between the various parts, through which the enteric cavity is in communication with the exterior.

In spite of this fact I have often found pieces distended with water, before any closure of the ends by new tissue has occurred. A series of experiments in which the body-wall was sectioned transversely at some level and the oral portion, still bearing tentacles and disc intact, was used, will serve to illustrate this point. In every case collapse of the tentacles and body occurred immediately after section, owing to the escape of water from the enteron, but very frequently the whole oral piece including the tentacles was again distended with water in less than an hour. Examination of the aboral cut end in such cases showed that inrolling and approximation of the margins had occurred, but frequently distinct spaces between the wrinkles could be observed opening into the enteron. If the end were spread open with

needles collapse occurred at once, but was followed by renewed extension in a short time. Pieces with tentacles and disc intact show these changes much better than others, since the phenomena of distention and collapse are especially conspicuous in the tentacles. Moreover, in these pieces the presence of the mouth permits much more rapid entrance of water than is possible in pieces with oral end removed, since in these latter there is no apparatus for forcing the water into the enteron. The rapid distention of pieces under the conditions described is made possible only by the ectodermal slime secretion which under normal conditions forms the tube.

The manipulation incidental to section of the body and the stimulus of the cut itself cause a rapid secretion of this slime during the operation and for some time after. The secretion is tenacious even when first formed and clings closely to the body.

After inrolling has occurred at a cut surface only ectoderm is visible from without and where different parts of the inrolled margins are in contact the contact is usually in part ectodermal. The slime is secreted over this inrolled portion and forms a tenacious coating which closes all the crevices between the inrolled portions of the body-wall. Thus, so far as the escape o water in appreciable quantities is concerned, the cut end may be closed within a short time after section, almost as soon, in fact, as the inrolling is completed.

If the piece is left undisturbed the slime accumulates and the closure becomes more and more complete until finally the thin membrane of new tissue constitutes the definitive closure. If at any time before the definitive closure the slime be carefully removed with needles without causing violent contraction or changes in form of the piece, collapse will occur at once, showing clearly that the slime alone prevented the escape of the water.

The Growth of New Tissue from the Cut Surface.

The closure of the ends by new tissue was briefly described in the first paper of the present series, but the conditions which determine it were not discussed.

As described, the course of the process at both oral and aboral ends in typical regeneration is as follows: First the appearance of

a thin membrane of new tissue between those regions of the cut surface which are sufficiently approximated; the growth of this membrane until the whole opening is closed; the increase in size of the area of new tissue as the piece becomes distended.

During the course of my experiments it was found that certain definite conditions are necessary for this growth of new tissue.

Mention was made of the fact that the new tissue appears first in the folds and wrinkles where two cut surfaces are most closely in contact and that from these regions it extends until closure is complete. In pieces rolled spirally (Figs. 17, 19, 24) or in any such manner that the cut surfaces are not brought into contact no appreciable growth of new tissue occurs; the cut edges heal, but may remain without further change for months.

Thus, in these spirally or transversely rolled pieces typical regeneration of new tissue from the cut surface does not occur. Moreover, this is true of all cases in which there is no approximation or contact of two cut surfaces or parts of a cut surface. Never is a thin membrane of new tissue found growing out from a cut surface and without other connections. When present it always connects two cut surfaces or the two sides of a fold where different regions of the cut surface have been approximated.

This is a point of considerable importance; indicating as it does that there is nothing in the cut surface itself which initiates regeneration, the necessary condition being found rather in the relations of different cut surfaces or their parts. *Never* do we find regeneration of the body-wall occurring in the manner represented in the diagram, Fig. 25, as a continuation with free margin of the old tissue. New tissue arising from cut surfaces always appears between two cut surfaces which are in contact or closely approximated as in Fig. 2. These surfaces become united by new tissue which then increases in amount under certain conditions, thus forming a thin membrane connecting the two parts of the cut surface. In the ordinary closure of the end of a cylindrical piece the new tissue first appears, as has been noted, in the folds and wrinkles where parts of the cut surface are closely approximated (Figs. 6–8, '03*a*), but from this it spreads rapidly until the whole space is covered and the end closed (Fig. 9, '03*a*).

The process is briefly as follows: After exposure of a cut surface some slight proliferation occurs which results in healing unless another cut surface be so near that the cells arising from both are in contact; if this is the case then organic union between the two cut surfaces is rapidly established. In the closure of the ends of cylindrical pieces this process is usually completed in a few days, but in certain other cases it may proceed much more slowly. For example, in pieces which are split down one side (Fig. 10) and in which both of the longitudinal cut margins roll inward as in Fig. 11, the process of closure often requires two months or more for completion. In such cases the longitudinal cut margins usually roll inward so far that they are not in contact. At one or both of the ends, however, the closure may occur in nearly the typical manner. From the end the new tissue begins to grow along the longitudinal cut, and as it grows actually draws the cut edges together to a certain extent. The process may be compared for the sake of illustration to that of sewing up the longitudinal slit in the piece from one or both ends. If we take, for example, a case where two cut surfaces are in contact at one point and diverge at an acute angle from this point, we find that the growth of new tissue always begins at the point of contact. From this point growth and the formation of a thin membrane continue for a certain distance along the diverging cut surfaces, the extent of the membrane depending in a given species on the angle of divergence of the surfaces. This thin membrane is itself somewhat elastic and so tends to approximate the cut surfaces in greater or less degree unless opposed by other conditions. The approximation of the surfaces renders possible a further extension of the membrane between them, and so the process continues unless at some point the cut surfaces are so situated that the elasticity of the new tissues is insufficient to bring them into contact, or near enough to permit the extension of the thin membrane between them. In such a case the process of closure must cease, as often occurs. That this is actually what occurs I have convinced myself by repeated examination of specimens cut in such manner that at least some parts of the cut surfaces were not in contact while others were. The diagrammatic Figs. 26–30 will serve to illustrate the process. Fig. 26 shows

a cylindrical piece slit down one side and represented as cut across obliquely to show the separation of the cut surfaces; the cut surfaces have rolled inward and the oblique section at the lower end of the figure shows that the inrolling along the longitudinal cut is so great that the cut surfaces are not in contact. The growth of new tissue and closure begins at the oral end (it may begin at the aboral end also) where the cut surfaces are much more closely approximated and the numerous folds afford

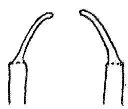

FIG. 25.

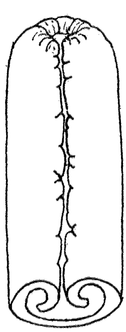

FIG. 26.

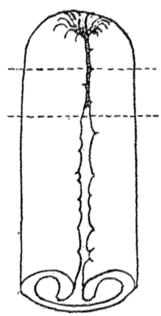

FIG. 27.

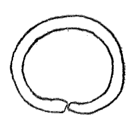

FIG. 28.

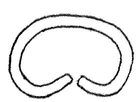

FIG. 29.

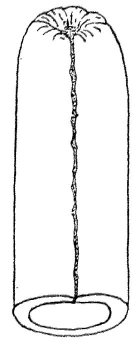

FIG. 30.

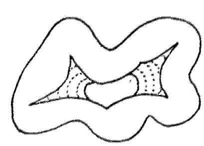

FIG. 31.

various points of contact. From this region it gradually extends along the longitudinal cut, drawing the cut surfaces together as it proceeds, as is shown by the two transverse sections, Figs. 28 and 29, Fig. 28 from a level where union has already occurred (the upper transverse line in Fig. 27) and Fig. 29 from a level just beyond where the cut surfaces have united (the level of the lower transverse line in Fig. 27). In Fig. 28 the margins have been drawn together and united, in Fig. 29 they are not yet in contact but are nearer together than at the level of the oblique section at the lower end of Fig. 27. In Fig. 30 the closure is

finally complete. In these figures only one end has been shown; usually, however, closure proceeds from both ends, and the middle portions are the last to become united.

It is evident that in a spirally or transversely rolled piece this process can never occur, for even if it begins in some fold or local approximation of the cut surfaces it cannot continue, because approximation of the cut surfaces to a degree sufficient to permit their union by new tissue is impossible as long as the piece remains spirally or transversely rolled.

Description of all possible cases of closure of pieces cannot of course be attempted. Results depend so largely on chance, that every piece affords, it might almost be said, a different solution of the problem. The examination of hundreds of pieces has, however, convinced me that the essential features of the process are those described above.

The cut surfaces appear to remain capable of giving rise to new tissue for an indefinite period. Often closure of pieces slit open longitudinally is completed only two or three months after section; yet during all this time the cut surface retains the power of producing new tissue under proper conditions, though it has no power to produce anything more than the proliferation connected with healing, provided it is not in contact with another surface. Union is as complete and perfect, though perhaps not as rapid, when it occurs two months or more after section as when it occurs within a few days.

When we compare *C. solitarius* and *C. membranaceus* we find that in the latter species the thin membranous growth of new tissue if once begun between cut edges in contact, may continue until it forms a connecting membrane between widely separated cut surfaces; in *C. solitarius*, on the other hand, the membrane is incapable of bridging over spaces so wide; the new tissue ceases to extend long before a point is reached where the cut surfaces are so widely separated. This difference is so marked that it raises the question as to whether there is a fundamental difference in the conditions and method of growth of new tissue in the two species. Figs. 6–9 of paper I. ('03*a*) and Fig. 31 of the present paper (an aboral end) illustrate the closure of openings in *C. membranaceus*. In Fig. 31 the new tissue which is

growing over the opening is in two parts which are advancing to meet near the middle. The concave free margin of both portions is noticeable. The dotted lines represent various stages in the growth of the new tissue. It is evident that it appeared first in the angles where two cut surfaces were in contact or very closely approximated. In *C. solitarius* closure by new tissue of only very much smaller pieces is possible. I believe the explanation of this difference is to be found in the different quality of the thin membrane of new tissue in the two cases. In *C. membranaceus* it is much thicker, more resistant, and less easily ruptured than in *C. solitarius*. The new tissue arises at a region where the cut surfaces are close together and may extend from this to regions where they are more widely separated. As was shown above, it exerts a certain degree of tension on the parts connected by it. As the distance between the cut surfaces increases a point may finally be reached where the tension is equal to the cohesive power of the tissue elements. Beyond this the new tissue cannot extend. In *C. solitarius* this limit is attained with a slight separation of the cut surfaces, while in *C. membranaceus* the new tissue is capable of resisting much greater tension and so of extending over wider spaces.

The membrane extending between the two cut surfaces may be compared with a fluid film bounded by lines diverging at an acute angle. The film extends a certain distance from the apex of the angle, this distance being determined with a given fluid by the size of the angle. The free margin of the film is always concave toward the opening of the angle. So long as the relation between cohesion and adhesion remains the same and the angle does not change the film can never extend beyond a certain point, since the surface-tension will cause rupture. As the angle and surface-tension decrease or as the adhesion increases the film will spread. If the arms of the angle are sufficiently pliable or capable of movement they may be drawn together by the surface-tension of the fluid, and thus permit further extension of the film.

In *Cerianthus* the thin membrane of new tissue which may be compared to the fluid film, arises at the apex of the angle, *i. e.*, where the two cut surfaces are in contact. The membrane extends along the diverging surfaces to a certain point. Its free margin is always concave (Fig. 31, also Figs. 6–8 ; '03*a*). The

distance to which the membrane spreads between the surfaces depends upon its composition and the degree of divergence of the surfaces, just as in the case of the fluid film. The thicker membrane with greater resistance will grow farther just as the film with less surface-tension will spread farther, other things being equal. Even the elasticity of the newly formed membrane is paralleled by the tension to which the fluid film is subjected. In both cases the margins of the space may be approximated by this tension and thus permit further spreading of the connecting film or membrane.

The illustration of the fluid film has been employed primarily as an analogy. It is not to be supposed that the thin membrane growing between two cut surfaces behaves in all respects like a fluid film extending across an angle. Yet the close parallelism between the two series of phenomena must raise the question as to whether after all the growth of new tissue from a cut surface in *Cerianthus* may not be, at least to a large extent if not entirely, determined by the laws which govern the behavior of fluids. The following facts point toward this conclusion : except so far as healing of a cut surface is concerned new tissue arises from a cut surface only when it is in contact with another, the thin membrane of new tissue which is under tension spreads between diverging cut surfaces to a certain point, beyond which no growth occurs, unless the surfaces are brought nearer together ; the point where growth ceases differs in different species, depending on the quality of the membrane ; the cut surfaces may themselves be approximated by the tension of the membrane and so further growth of the membrane made possible ; the free margin of the membrane is always concave in the direction of growth, *i. e.*, the margins of the membrane extend further than its middle region. In all of these respects the thin membrane and the fluid film behave similarly. The conclusion is at least probable that the similarity in behavior is due to the fact that similar conditions are present. I think it probable, therefore, that the appearance and growth of new tissue from the cut surfaces of the body of *Cerianthus* is governed, at least to a large extent, by the laws of capillarity. Of course the cellular structure of the tissue may complicate conditions, and the thickening and structural differentiation which

occur in the membrane after its formation bring into play other factors. These need not be considered here, however. Certainly the phenomena are far from being adaptive or teleological in any sense although the closure of the cut might appear at first glance to be an adaptation. It is difficult at present to see how they can be due to anything except simple physical conditions, though it is possible that increased knowledge may afford another explanation. Provisionally then we may regard the delicate thin membrane which appears in the angles between cut surfaces as possessing some of the properties of a fluid and as subject, at least in large degree, to the laws of capillarity.

Whether these suggestions are correct or not, the two facts above mentioned are of great importance, viz., that regeneration of new tissue from cut surfaces occurs only when two surfaces are in contact, and that the new tissue cannot extend indefinitely between diverging cut surfaces but ceases at a certain point determined by the angle of divergence of the two surfaces and the (physical) quality of the membrane, *i. e.*, is different in different species. The only possible inference from these facts is that all conditions for regeneration are not given in the living tissues themselves, nor in these plus the normal environment as a whole, but that the formation of new tissue from a cut surface is probably dependent upon certain simple physical conditions similar to those which govern the existence of a liquid film between two diverging boundaries.

Healing of the cut surface does not require these conditions; for this the necessary conditions, which are very probably also primarily due to capillarity, are established by the cut itself. The same conditions are not, however, adequate for the formation of a membrane of new tissue from the cut surface.

In this case then the conditions for new growth and closure of a wound are to be found, not in the absence of a certain part, nor in the presence of a special stimulus at the cut surface, but in simple, external, physical relations of parts. Discussion of the bearing of these facts may be postponed to another time. Attention may be called, however, to the difficulty of reconciling these facts with the neo-vitalistic theories of life and especially with that of Driesch which is based upon the phenomena of form-regula-

tion and has adopted a modification of the Aristotelian idea of an entelechy as the basis of organic form.

Summary.

1. The inrolling of the margins and the closure of openings by contact of the inrolled margins is the result of the elasticity of the body-wall. This elasticity must be greater in the inner portions than in the outer portions, in order to produce the results observed. The facts indicate that the mesoglœa plays the most important part in this elastic contraction.

2. Openings between folds of the inrolled body-wall may be stopped by the ectodermal slime secretion. This method of closure often occurs in pieces before the formation of new tissue and permits the existence of considerable water-pressure in the enteron.

3. Contact or close approximation between two cut surfaces or parts of a cut surface is a necessary condition of the growth of new tissue from these surfaces. A single exposed cut surface may heal over but no further growth occurs from it.

4. The new tissue having arisen at a point of contact between two cut surfaces is capable of extending in the form of a thin membrane for a certain distance between diverging cut surfaces; the distance to which it extends is determined in a given species by the angle of divergence of the cut surfaces, and in different species by the thickness and quality of the membrane.

5. The new tissue rising between two cut surfaces behaves in certain respects as if subject to the laws of capillarity.

Hull Zoölogical Laboratory,
University of Chicago, September, 1903.

BIBLIOGRAPHY.

Child, C. M.

'03a Form-Regulation in Cerianthus, I. The Typical Course of Regeneration. Biol. Bull., Vol. V., No. 5, 1903.

'03b Form-Regulation in Cerianthus, II. The Influence of Position, Size and other Factors upon Regeneration. Biol. Bull., Vol. V., No. 6; Vol. VI., No. 1, 1903.

Loeb, J.

'91 Untersuchungen zur physiologischen Morphologie, I. Heteromorphose. Würzburg, 1891.